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Please find below and/or attached an Office communication concerning this application or proceeding.

The time period for reply, if any, is set in the attached communication.

DETAILED ACTION

Claim Rejections - 35 USC § 112

The following is a quotation of the second paragraph of 35 U.S.C. 112:

> The specification shall conclude with one or more claims particularly pointing out and distinctly claiming the subject matter which the applicant regards as his invention.

Applicants have traversed the rejection of record under 35 USC 112, second paragraph, on the grounds that the specification makes it clear that the mononuclear cell population comprises about 1-5% gamma delta T lymphocytes, and thus the method is intended to enrich the proportion of gamma delta T lymphocytes. Applicants assert the term 'transdifferentiation' is not used anywhere in the specification, and thus would not be considered by one of ordinary skill in the art.

In response, the rejection of record stands. The claim, as currently written, does not make it clear what proportion, if any, of mononuclear cell population are gamma delta T lymphocytes. Without such a disclosure of the starting cell composition, the method cannot be put into context. The limitation from the specification that the mononuclear cell composition comprises a specific proportion of gamma delta T lymphocytes cannot be read into the claims. Although the claims are interpreted in light of the specification, limitations from the specification are not read into the claims. See *In re Van Geuns*, 988 F.2d 1181, 26 USPQ2d 1057 (Fed. Cir. 1993).

Therefore claims 21, 23-25, 29-31 stand rejected under 35 U.S.C. 112, second paragraph, as being indefinite for failing to particularly point out and distinctly claim the subject matter which applicant regards as the invention.

In claim 21, it is not clear if the blood sample or cytapheresis sample initially contains gamma delta T lymphocytes, and the method is intended to enrich the proportion of gamma delta T lymphocytes within the sample; or if the biological preparation may contain any mononuclear cells, and the method is intended to involve transdifferentiation of various (non-gamma delta T lymphocytes) mononuclear cells into gamma delta T lymphocytes. As is, the claims do not currently make the intent of the method clear, or the steps necessary to achieve the method, if a transdifferentiation step is required such must be expressly claimed. Clarification is required.

Claim Rejections - 35 USC § 103

The following is a quotation of 35 U.S.C. 103(a) which forms the basis for all obviousness rejections set forth in this Office action:

> (a) A patent may not be obtained though the invention is not identically disclosed or described as set forth in section 102 of this title, if the differences between the subject matter sought to be patented and the prior art are such that the subject matter as a whole would have been obvious at the time the invention was made to a person having ordinary skill in the art to which said subject matter pertains. Patentability shall not be negatived by the manner in which the invention was made.

Applicants' arguments received with the response of 5/4/2008 have been fully considered, but are not found persuasive to overcome the rejection of record.

Applicants have traversed that the level of IL-2 used in the instant invention would have been obvious, based on the disclosure of Garcia et al. Applicants first assert Garcia et al is limited to pure populations of gamma delta T lymphocytes, whereas the instant claims are directed to populations of mononuclear cells of which 1-5% are gamma delta T cells. Second Applicants assert Garcia et al only show significant increase in cell numbers when 100 ng/mL of IL-2 was used (1630 U/mL), which is significantly out of the range currently claimed. Third, Applicants assert Garcia et al teach IL-2 is not a good cytokine to obtain gamma delta T cell

proliferation. Fourth, and finally, Applicants assert Belmant cannot be said to use 100 U/mL of IL-2, as they provide the IL-2 in sequential dosages, and thus the first 50 U/mL would have been consumed prior to addition of the second dose.

Applicants' arguments are not found persuasive for the following reasons:

In response to Applicants' first argument, it is submitted that the claims are not limited to culturing a mononuclear cell population comprising only 1-5% of gamma delta T lymphocytes. The claims do not define the cell content of the mononuclear cells culture at all, thus Applicants are arguing limitations not in the current claims.

In response to Applicants' second argument, it is respectfully submitted that Garcia et al clearly report an increase in gamma delta T lymphocyte numbers in cultures comprising 10 ng/mL of IL-2 (~163 U/mL IL-2) (See Figure 2). The fact that further increase in cell number may be achieved with increased levels of IL-2 does not negate the fact that Garcia et al clearly show cultures treated with 10 ng/mL IL-2 exhibited increased cell proliferation.

In response to Applicants' third argument, the fact that Garcia et al report IL-15 is superior to IL-2 does not equate to a teaching that IL-2 is 'not a good cytokine to obtain gamma delta T cell proliferation' (Response, Pg 6). A reference merely not teaching every limitation does not constitute teaching away by that reference. See *In re Grasselli* 713 F.2d 731, 741, 218 USPQ 769, 777 (Fed. Cir. 1983).

In response to Applicants' fourth argument, this point is well taken, and the rejection has been amended to remove this point, but it remains that Garcia et al suggest ~163 U/mL of IL-2, which is falls within the claimed range.

Applicants have also traversed that the duration of culture period may be routinely extended to periods greater than 7 days, since Skea et al indicate maximum proliferation of the cells would have occurred by day 7.

In response, it is respectfully submitted that the rejection of record specifically addresses this point: Example 10 of Belmant et al involves culturing the cells for 8 days (which, itself, may be considered to read on "about 10 days". Yet, it is noted that Example 14 reports in vitro culturing of lymphocytes for a period of 10 days (See Belmant et al, col. 25, Table II). Therefore, the lymphocytes may be maintained in culture for up to at least 10 days, and presumably longer, as there is no requirement that the culture continues to increase in proportion of gamma delta T lymphocytes over time; once the culture has reached its maximum percentage of gamma delta T lymphocytes, it would be reasonably expected that the culture may simply be maintained.

Therefore the rejection of record is maintained as appropriate over the claims as currently amended.

Claims 21, 23-25 and 29-31 stand rejected under 35 U.S.C. 103(a) as being unpatentable over Belmant et al (US Patent 6,660,723), in view of Skea et al (Journal of Hematotherapy and Stem Cell Research, Oct 2001), Garcia et al (Journal of Immunology, 1998) and Valeri (Blood Banking and the Use of Frozen Blood Products, 1976).

Belmant et al teach a method for enriching the concentration of gamma delta T lymphocytes (Tγ9δ2 lymphocytes) in a cell sample (which reads on what Applicants are calling preparing a gamma delta T lymphocyte composition).

Example 10 of Belmant et al is relied upon: Belmant culture 1 mL of a blood extract comprising 10^6 T lymphocytes in culture medium comprising inactivated human serum, 0.1-100 nM BrHPP or IHPP, and 50 U/mL of IL-2. After 4 days Belmant et al report adding another 50 U/mL of IL-2 to the culture (See Belmant et al, col. 2, ln 44-65 & specifically Example 10, col. 22, ln 1-34). The proportion of delta gamma T lymphocytes was reported as increasing from 1-5%, in the initial sample, to 10% to greater than 50% in the presence of BrHPP and to 10% to about 80% in the presence of IHPP (See Fig. 1).

Belmant et al differ from the instant invention in that they do not disclose the same specific culture parameters recited in the instant claims, including the original cell count, cell density during culture, culture duration, or concentration of the cytokine. However, it is maintained that it would have been well within the purview of one of ordinary skill in the art to optimize the duration of the culture period, the initial cell number, the culture density during culture, and the concentration of the IL-2 to those values recited in the instant claims, as each of the parameters was recognized in the art as acceptable values for T lymphocyte cell culture, and/or were recognized as result effective variables, which would have been routinely optimized by the artisan of ordinary skill.

With regards to the cell count of the initial cell sample, the current claims require an initial sample comprising at least 50 million mononuclear cells. The sample of Belmant et al only comprises 1 million mononuclear cells (10^6 T lymphocytes, see Belmant et al, col. 22, ln 8). However it is maintained that, in the field of cell culture, the starting cell count is generally

recognized to be a result effective variables that directly affect the final cell number produced by the culture. Therefore, the culture of Belmant et al may be 'scaled up' to involve 50-100 mL of sample, which would contain 50-100 million cells. The scaling up of the original sample size would produce a correspondingly greater number of delta gamma T lymphocytes in the end, including over 100 million functional and viable gamma delta T cells.

With regards to the concentration maintained throughout the culture period, Belmant et al reports providing the cells at a concentration of 1 x 10^6 cells/mL (See Belmant et al, col. 22, ln 8-9), which is less than 5 x 10^6 cells/mL. Presumably Belmant et al maintain the cell culture at this density, though they do not explicitly state such. However, Skea et al is relied upon to show the art taught cultures of mononuclear cells for enrichment of gamma delta T lymphocytes were routinely maintained at densities of 1 x 10^5 cells/mL (See Skea et al, Pg. 526, col. 1, "T Cell Culture"). Therefore, it is submitted that one of ordinary skill in the art, at the time the invention was made, would have been able to determine an appropriate concentration at which to maintain the cell culture of Belmant et al, the concentration being less than 5 x 10^6 cells/mL.

With regards to the duration of culture, while Example 10 involves culturing the cells for 8 days (which, itself, may be considered to read on "about 10 days"), it is noted that Example 14 reports in vitro culturing of lymphocytes for a period of 10 days (See Belmant et al, col. 25, Table II). Therefore, the lymphocytes may be maintained in culture for up to at least 10 days, and presumably longer, as there is no requirement that the culture continues to increase in proportion of gamma delta T lymphocytes over time; once the culture has reached its maximum

percentage of gamma delta T lymphocytes, it would be reasonably expected that the culture may simply be maintained.

With regards to the concentration of the IL-2 provided in the culture, while Belmant et al disclose using 50-100 U/mL of IL-2, the concentration of the cytokines was recognized as a result effective variable that would have been routinely optimized by the artisan of ordinary skill. In support, see Garcia et al. Garcia et al measure gamma delta T cell response to IL-2, and report that in three out of four cell clones tested, the proliferation rate of gamma delta T cells increased with increasing concentrations of IL-2 (See Garcia et al, Fig. 2). In all four cell clones tested, an increase was achieved with 10 ng/mL of IL-2 (10 ng/mL = approximately 163 U/mL, using conversion factor of 1 ng = 16.3 U). Therefore, use of IL-2 at a concentration of 163 U/mL was recognized as a concentration at which gamma delta T lymphocytes are successfully activated, and thus optimization to such a range would have been within the purview of one of ordinary skill in the art, as the general range was taught by the art (i.e. Garcia et al). Generally, differences in concentration will not support the patentability of subject matter encompassed by the prior art unless there is evidence indicating such concentration is critical or produces unexpected results. Where the general conditions of a claim are disclosed by the prior art it is not inventive to discover the optimum or workable ranges by routine experimentation, See *In re Aller,* 220 F.2d 454, 456, 105 USPQ 233, 235 (CCPA 1955).

Finally, while Belmant et al teaches the gamma delta T lymphocytes can be from a blood sample or blood extract, they do not specifically teach separating whole blood by cytapheresis prior to the culture method described above, nor do they teach using previous frozen samples of

blood. However, at the time the invention was made it would have been well within the purview of one of ordinary skill in the art to obtain a blood sample at a first point in time, separate the whole blood sample by cytapheresis into individual components, freeze the individual components by appropriate means known in the art, and then at later time, thaw the desired platelet component for use in the method of Belmant et al. In support see Valeri; Valeri teaches the basic protocols for obtaining, separating and storing blood. Valeri teaches cytapheresis allows separation of specific components of blood from whole blood (See Valeri, Pg. 1, col. 1). Separating the platelets via 'plateletpheresis' removes the majority of red blood cells and plasma components, thereby increasing the proportion of gamma delta T lymphocytes present in the initial cell sample, which is desirable in the method of Belmant et al, as it would ensure a higher proportion of gamma delta T lymphocytes for culture and expansion, resulting in a greater end cell count. Additionally, Valeri teaches methods for frozen storage of separated platelets (See Valeri, Pg 297), which is desirable when the blood sample must be stored for a period of greater than 4 hours prior to use in the method of Belmant et al (See Valeri, Pg. 6, col. 1). One would expect success separating the desired platelet component via cytapheresis and storing the separated components in a frozen state for future use, because such methods and procedures are common in the art of hematology, as demonstrated by the teachings of Valeri.

Therefore the invention as a whole would have been *prima facie* obvious to one of ordinary skill in the art at the time the invention was made.

Claims 21, 23-25 and 29-31 stand rejected under 35 U.S.C. 103(a) as being unpatentable over Espinosa et al (Journal of Biological Chemistry, 2001), in view of Skea et

al (J Hematotherapy & Stem Cell Res, 2001), Garcia et al (Journal of Immunology, 1998) and Valeri (Blood Banking and the Use of Frozen Blood Products, 1976).

Espinosa et al sought to identify a synthetic activator of gamma delta T lymphocytes that has comparable immunostimulatory activity as natural phosphoantigens; Espinosa et al discovered BrHPP enabled immunostimulation of human gamma delta T lymphocytes (See Espinosa et al, abstract).

Espinosa et al first perform a control run using the known, natural phosphoantigen 3-formyl-1-butyl-pyrophosphate (3fbPP); peripheral blood lymphocytes were cultured at an initial concentration of 10^6 cells/mL in the presence of 10nM 3fbPP and 100 U/mL IL-2 for a 15 day period (See Espinosa et al, Pg. 18338, col. 1). Espinosa et al report significant expansion of the gamma delta T lymphocytes, including compositions comprising greater than 95% TCR Vδ2 positive cells (gamma delta T lymphocytes) (See Espinosa et al, Pg. 18338, col. 2).

Espinosa et al then perform an experimental run using several different concentrations (12.5, 25, 100 nM) of BrHPP as the activator instead of the natural 3fbPP (See Espinosa et al, Pg. 18340, col. 1-2 & Fig. 4).

Espinosa et al do not specifically describe the culture conditions of the experimental run, while they do state that peripheral blood cells were used, they are silent on the initial cell count, the length of the culture period, and whether or not IL-2 was added to the culture. However, it appears the culture conditions for the experimental run were identical to the conditions of the control run: 10^6 cells/mL were present in initial culture as well as 100 U/mL of IL-2, and the culture was maintained for 15 days. One of ordinary skill in the art would assume that for results to be comparable between the immunostimulatory activity of the 3fbPP and BrHPP, the culture

conditions were identical. Therefore, in the absence of evidence to the contrary, it is assumed Espinosa et al performed a method for activation of a gamma delta T lymphocyte composition comprising culturing peripheral blood lymphocytes (PBL) in the presence of BrHPP (a synthetic activator of gamma delta T lymphocytes) and IL-2.

However, even if the culture conditions described for the control run were not duplicated in the experimental run, it would have been well within the purview of one of ordinary skill in the art to optimize the duration of the culture period, the cell density before and during culture, and the concentration of the activator compounds (BrHPP and IL-2) to those values recited in the instant claims, as each of the parameters was recognized in the art as acceptable values for T lymphocyte cell culture, or were recognized as result effective variables, which would have been routinely optimized by the artisan of ordinary skill.

With regards to the cell count of the initial cell sample, the current claims require an initial sample comprising at least 50 million mononuclear cells. While Espinosa et al disclose the concentration of the initial sample (10^6 cells/mL) they do not disclose the volume provided. However it is maintained that, in the field of cell culture, the starting cell count is generally recognized to be a result effective variables that directly affect the final cell number produced by the culture. Therefore, the sample of Espinosa et al may be 'scaled up' to involve 50-100 mL of sample, which would contain 50-100 million cells. The scaling up of the original sample size would produce a correspondingly greater number of delta gamma T lymphocytes in the end, including over 100 million functional and viable gamma delta T cells.

With regards to the concentration maintained throughout the culture period, Espinosa et al reports providing the cells at a concentration of 1 x 10^6 cells/mL (See Espinosa et al, Pg 18338, col. 1, "Cell Culture"), which is less than 5 x 10^6 cells/mL. Presumably Espinosa et al maintain the cell culture at this density, though they do not explicitly state such. However, Skea et al is relied upon to show the art taught cultures of mononuclear cells for enrichment of gamma delta T lymphocytes were routinely maintained at densities of 1 x 10^5 cells/mL (See Skea et al, Pg. 526, col. 1, "T Cell Culture"). Therefore, it is submitted that one of ordinary skill in the art, at the time the invention was made, would have been able to determine an appropriate concentration at which to maintain the cell culture of Espinosa et al, the concentration being less than 5 x 10^6 cells/mL.

With regards to the concentration of the IL-2 provided in the culture, while Belmant et al disclose using 50-100 U/mL of IL-2, the concentration of the cytokines was recognized as a result effective variable that would have been routinely optimized by the artisan of ordinary skill. In support, see Garcia et al. Garcia et al measure gamma delta T cell response to IL-2, and report that in three out of four cell clones tested, the proliferation rate of gamma delta T cells increased with increasing concentrations of IL-2 (See Garcia et al, Fig. 2). In all four cell clones tested, an increase was achieved with 10 ng/mL of IL-2 (10 ng/mL = approximately 163 U/mL, using conversion factor of 1 ng = 16.3 U). Therefore, use of IL-2 at a concentration of 163 U/mL was recognized as a concentration at which gamma delta T lymphocytes are successfully activated, and thus optimization to such a range would have been within the purview of one of ordinary skill in the art, as the general range was taught by the art (i.e. Garcia et al).

Generally, differences in concentration will not support the patentability of subject matter encompassed by the prior art unless there is evidence indicating such concentration is critical or produces unexpected results. Where the general conditions of a claim are disclosed by the prior art it is not inventive to discover the optimum or workable ranges by routine experimentation, See *In re Aller,* 220 F.2d 454, 456, 105 USPQ 233, 235 (CCPA 1955).

With regards to the concentration of the cytokine IL-2, while Espinosa et al teach the concentration of the IL-2 to be 100 U/mL (See Espinosa et al, Pg. 18338, col. 1) the concentration of the cytokines was recognized as a result effective variable that would have been routinely optimized by the artisan of ordinary skill. In support, see Garcia et al. Garcia et al measure gamma delta T cell response to IL-2, and report that in three out of four cell clones tested, the proliferation rate of gamma delta T cells increased with increasing concentrations of IL-2 (See Garcia et al, Fig. 2). In all four cell clones tested, an increase was achieved with 10 ng/mL of IL-2 (10 ng/mL = approximately 163 U/mL, using conversion factor of 1 ng = 16.3 U). Therefore, use of IL-2 at a concentration of 163 U/mL was recognized as a concentration at which gamma delta T lymphocytes are successfully activated, and thus optimization to such a range would have been within the purview of one of ordinary skill in the art, as the general range was taught by the art (i.e. Garcia et al).

It then naturally follows that because all of the above discussed variables directly effect the final gamma delta T lymphocyte cell count and concentration in the final composition, it would have been within the purview of one of ordinary skill in the art to optimize any and each

of the variables so as to produce a composition with any desired cell concentration and cell count, including at least 80% gamma delta T lymphocytes and at least 100 million cells. In the instant reference Espinosa et al report a final gamma delta T lymphocyte population which comprises approximately 63% of total lymphocytes (See Espinosa et al, Fig. 4a); alternatively, Espinosa et al teach the concentration of BrHPP directly effects the final gamma delta T lymphocyte count (See Pg. 18340, col. 2 & Fig. 4B). Thus one would have expected success optimizing the cell count and gamma delta T lymphocyte cell proportion because these variables are directly controlled by the culture steps and parameters discussed above, particularly the concentration of BrHPP; it would be well within the purview of one of ordinary skill in the art to optimize the culture conditions (adjust time between passages, adjust concentration of activation agents) in order to create a gamma delta T lymphocyte composition with the desired cell numbers and proportions.

Finally, while Espinosa et al teach use of peripheral blood lymphocytes, they do not specifically teach separating whole blood by cytapheresis prior to the culture method described above, nor do they teach using previous frozen samples of blood. However, at the time the invention was made it would have been well within the purview of one of ordinary skill in the art to obtain a blood sample at a first point in time, separate the whole blood sample by cytapheresis into individual components, freeze the individual components by appropriate means known in the art, and then at later time, thaw the desired platelet component for use in the method of Espinosa et al (Claims 23 and 25). In support see Valeri; Valeri teaches the basic protocols for obtaining, separating and storing blood. Valeri teaches cytapheresis allows separation of specific

components of blood from whole blood (See Valeri, Pg. 1, col. 1). Separating the platelets via 'plateletpheresis' removes the majority of red blood cells and plasma components, thereby increasing the proportion of gamma delta T lymphocytes present in the initial cell sample, which is desirable in the method of Espinosa et al, as it would ensure a higher proportion of gamma delta T lymphocytes for culture and expansion, resulting in a greater end cell count. Additionally, Valeri teaches methods for frozen storage of separated platelets (See Valeri, Pg 297), which is desirable when the blood sample must be stored for a period of greater than 4 hours prior to use in the method of Espinosa et al (See Valeri, Pg. 6, col. 1). One would expect success separating the desired platelet component via cytapheresis and storing the separated components in a frozen state for future use, because such methods and procedures are common in the art of hematology, as demonstrated by the teachings of Valeri.

Therefore the invention as a whole would have been *prima facie* obvious to one of ordinary skill in the art at the time the invention was made.